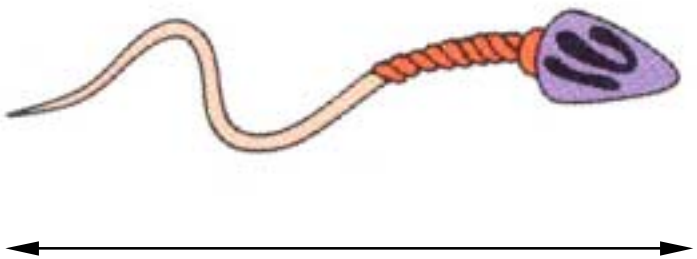
Analysis of Individual Cells and Endospores by Micro-Raman Spectroscopy

Anthony Esposito

Chemical Biology and Nuclear Sciences Division
Chemistry and Materials Science
Lawrence Livermore National Laboratory

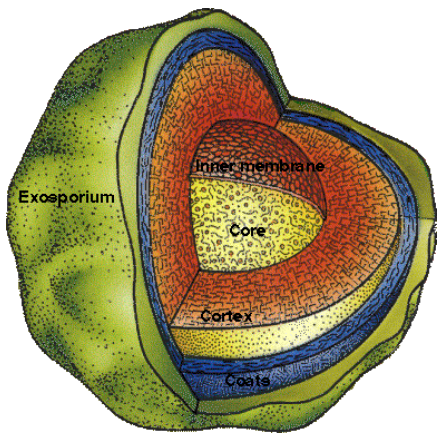
July 16, 2003

Our Goal is to Chemically Image and Characterize Individual Cells and Cell Components



characteristic size ~50 μm

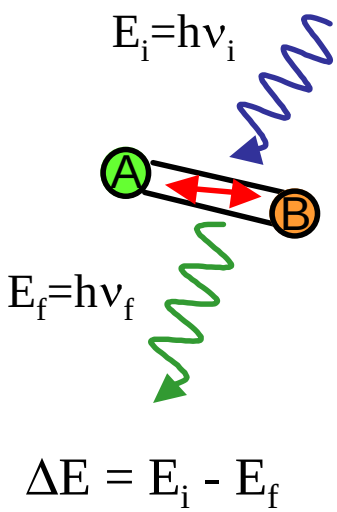
- "Large" cells (e.g. sperm cells)
  - Dimensions large relative to 1 μm laser spot
  - Have well-defined regions with known content (DNA, protein)
  - Good test case for applying micro-Raman to biological samples



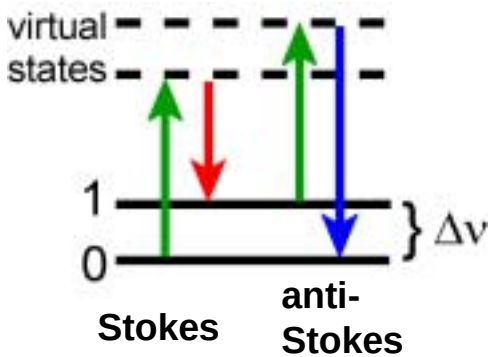
characteristic size ~1 μm

- "Small" cells (e.g. bacterial spores)
  - Dimensions similar to laser spot; entire spore is addressed at once
  - Difficult to distinguish between spores using only microscopy
  - Use micro-Raman to explore variations within a population and between species

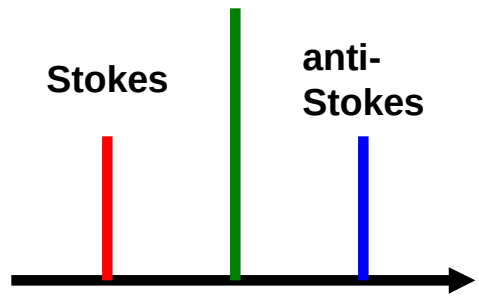
Raman Spectroscopy Uses Inelastic Light Scattering



vibrational levels



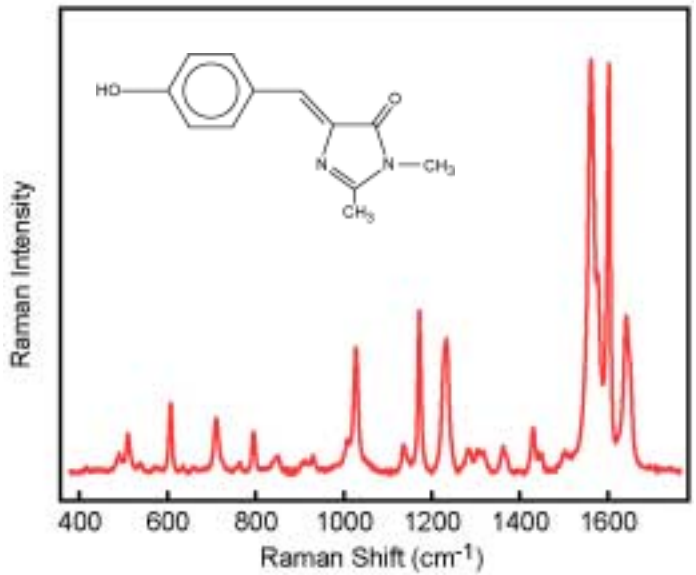
Raman spectrum



Energy, or Raman shift

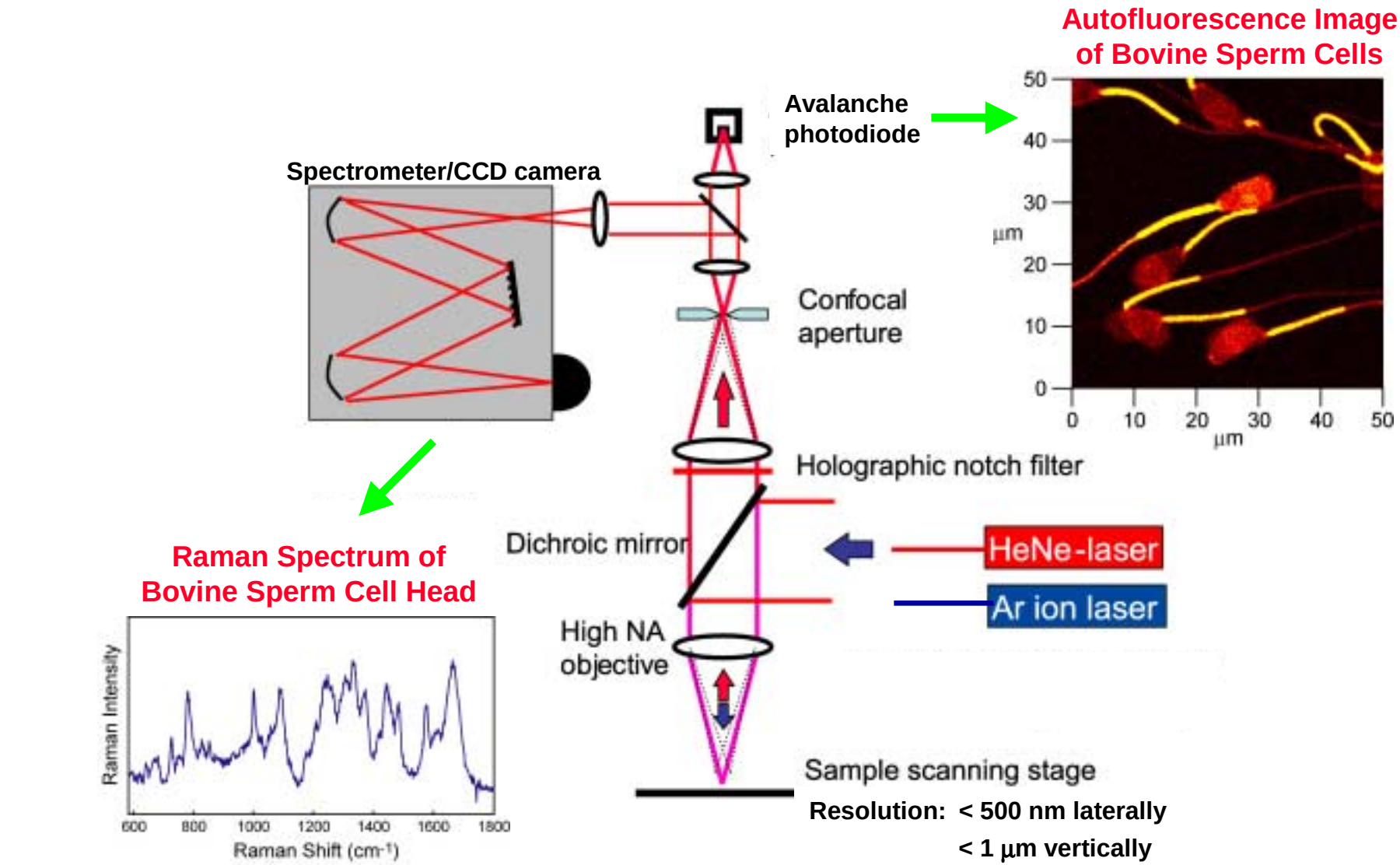
- **Net vibrational transition is achieved by inelastic scattering**
- **Spectrum of scattered light corresponds to vibrational frequencies**

Useful Characteristics of Raman Spectroscopy

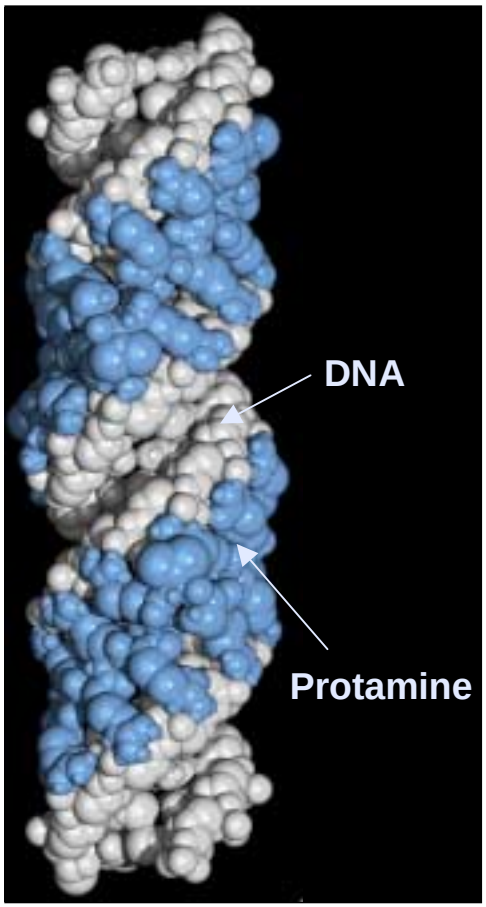


- **Provides fingerprint spectra (molecular identity, composition)**
- **Complementary to IR**
- **Sensitive to molecular conformation, environment**
- **Laser-based (easy to focus beam)**
- **Useful for biological applications**
  - **non-destructive**
  - **analytes do not photobleach**
  - **provides contrast without specific labeling**

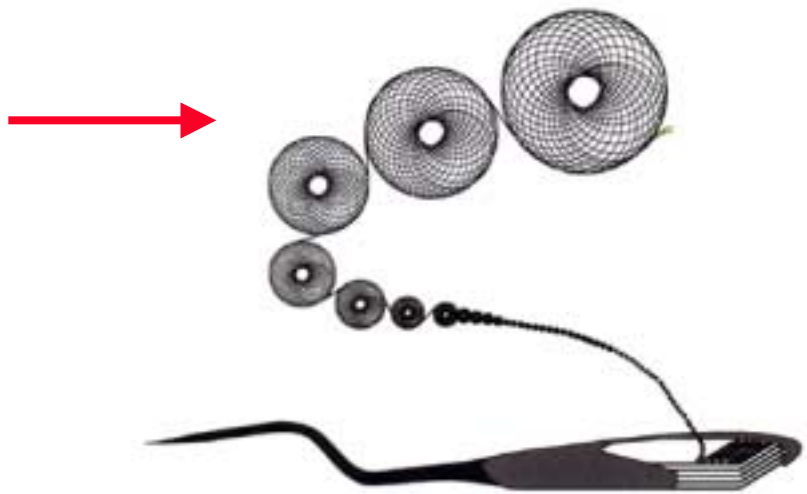
Micro-Raman Spectroscopy Provides Chemical Information with High Spatial Resolution



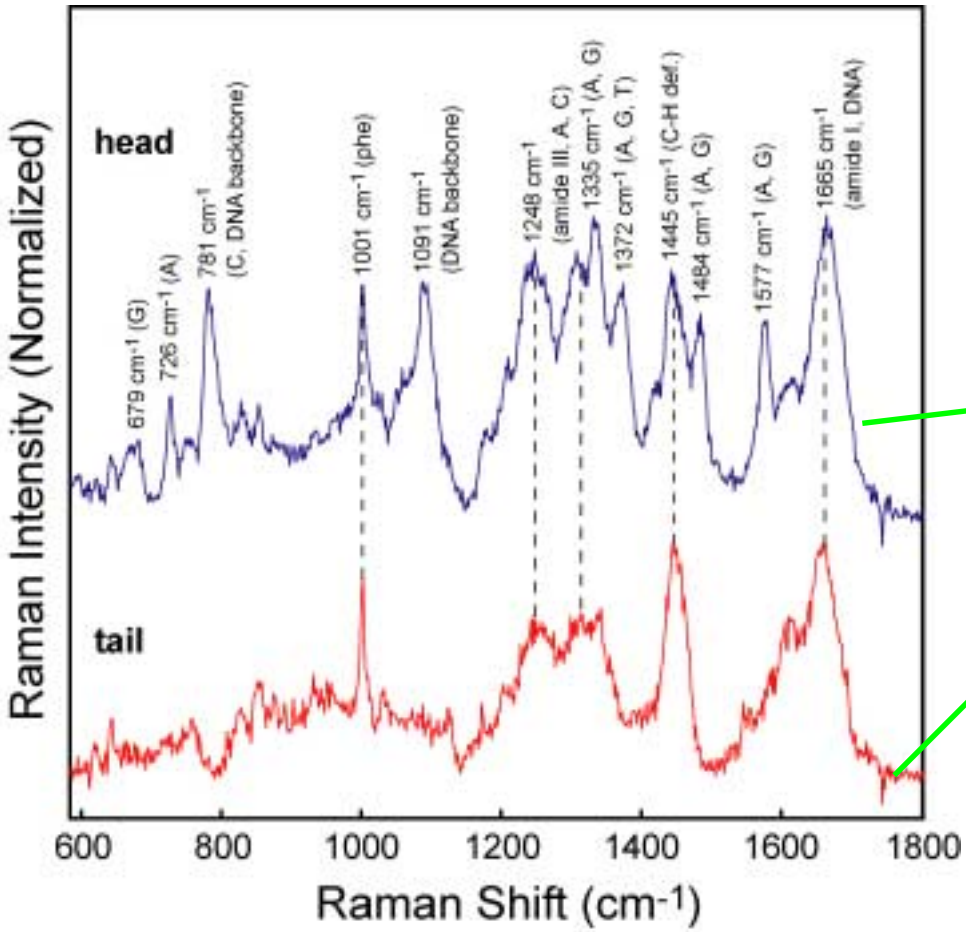
Characterizing Different Sections of Sperm Cells Is A Good Test Case for Micro-Raman Spectroscopy

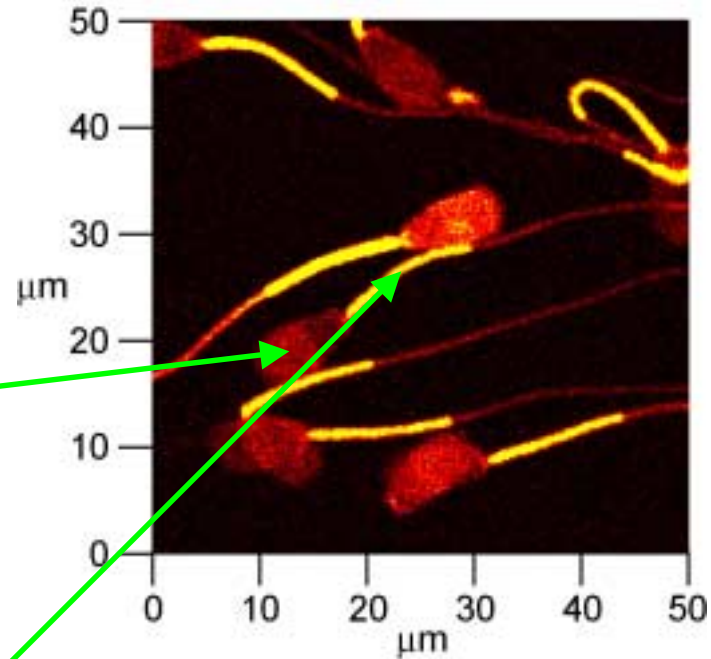


- Improperly packaged DNA is an indication of infertility
- Cell head primarily contains DNA and protein—can study relative amounts
- Cell tail contains mostly protein—provides contrast with head



Micro-Raman Spectra of Bovine Sperm Cells Provide Localized Chemical Information





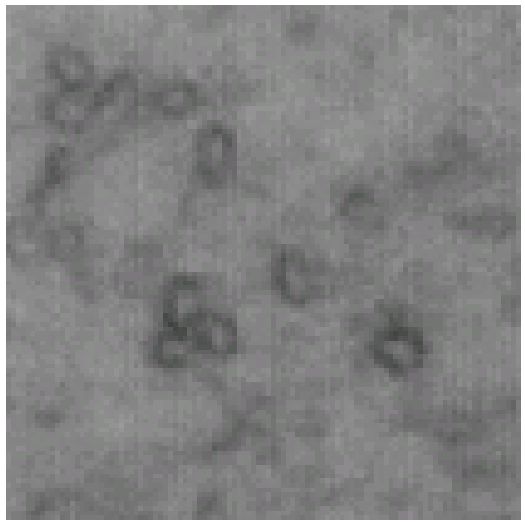
- **Spectra from head indicate protein and DNA**
- **Spectra from tail lack Raman scattering from DNA**

λ_{ex} = 488 nm

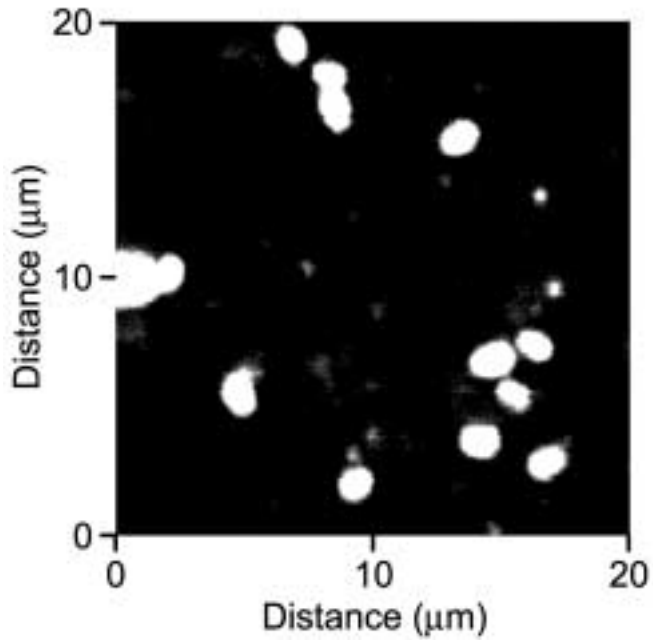
Use Chemical Information from Micro-Raman to Characterize Bacterial Spores

B. cereus

microscope

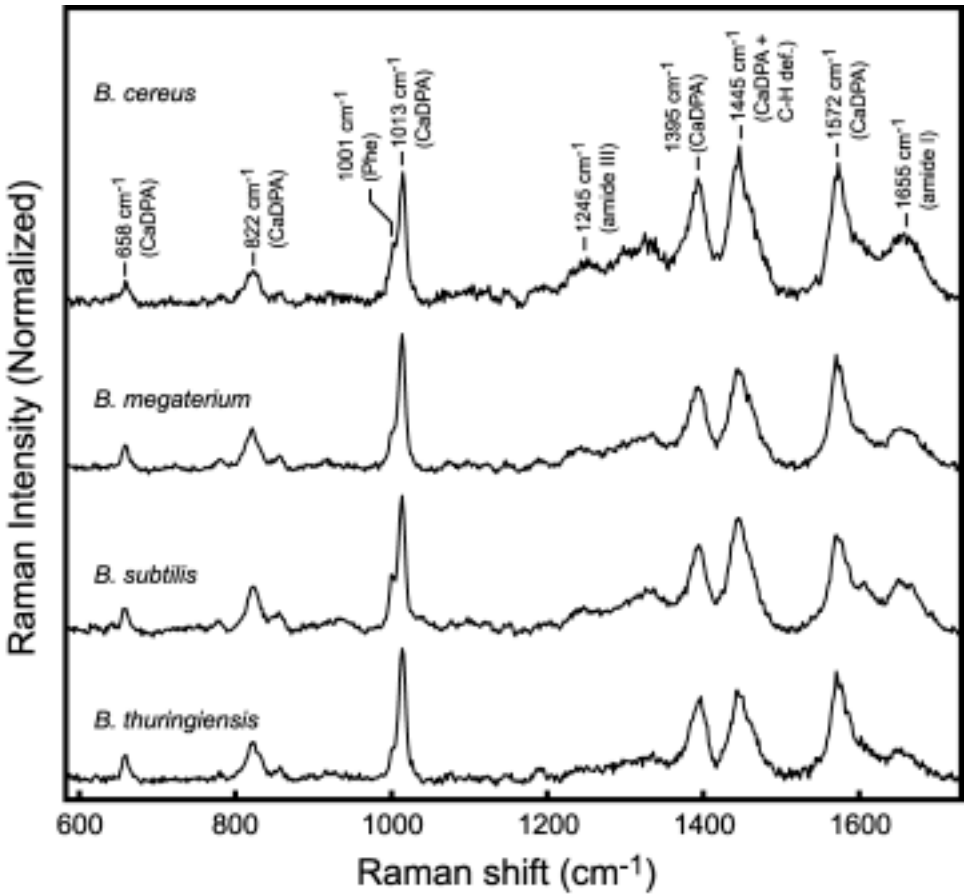


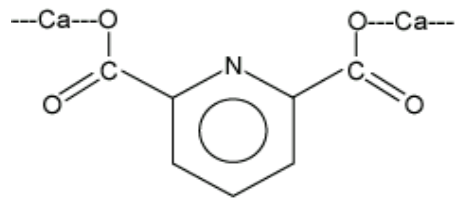
fluorescence



- **Identifying bacterial spores important for public health, security**
- **Spores ~1 μm in size, difficult to distinguish with microscopy**
- **Use micro-Raman to characterize individual spores from several *Bacillus* species**
- **See if distinct Raman marker bands can be found for different species**

Raman Spectra from *Bacillus* Spores Are Dominated by Calcium Dipicolinate





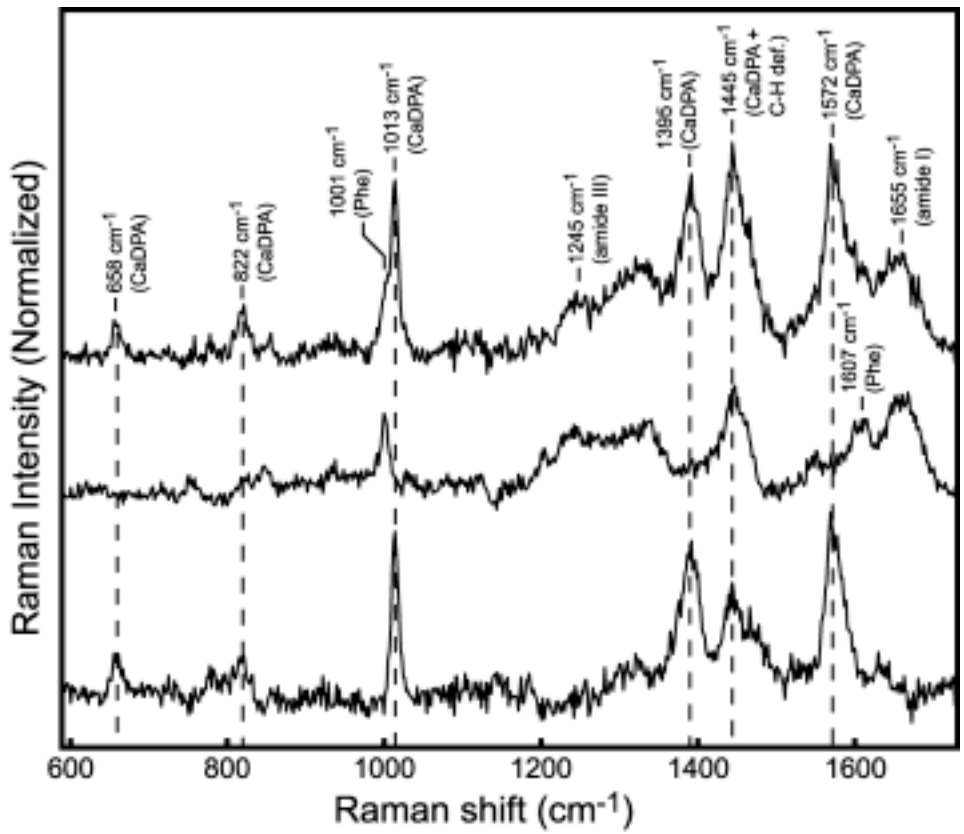
calcium dipicolinate (CaDPA)

- **Can obtain Raman spectra from single spores**
- **Spectra contain peaks from CaDPA and protein**
- **Little variability between different *Bacillus* species**

λ_{ex} = 488 nm

Esposito et al., *Applied Spectroscopy* **57**, 868 (2003)

Spores With Low Amounts of CaDPA Still Exhibit Raman Scattering from Protein



B. thuringiensis

λ_{ex} = 488 nm

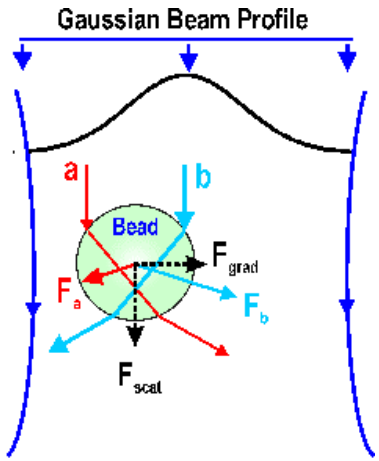
high CaDPA spore

low CaDPA spore

difference (high – low)

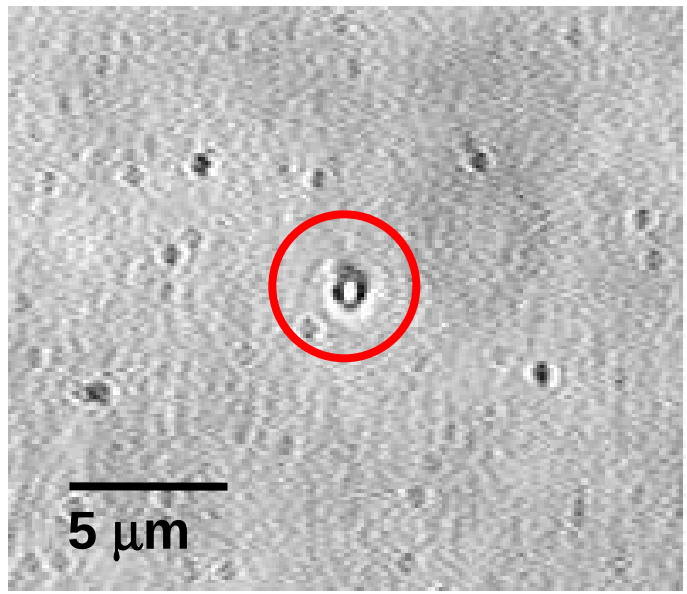
- **A small number of spores showed little CaDPA scattering, possibly from incomplete sporulation**
- **Can use micro-Raman to examine spore-to-spore variability**

We Have Recently Begun Optical Trapping In Order to Obtain Raman Spectra in Aqueous Environments



Optical trap

Optically-trapped *Bacillus* spore (B.g.)

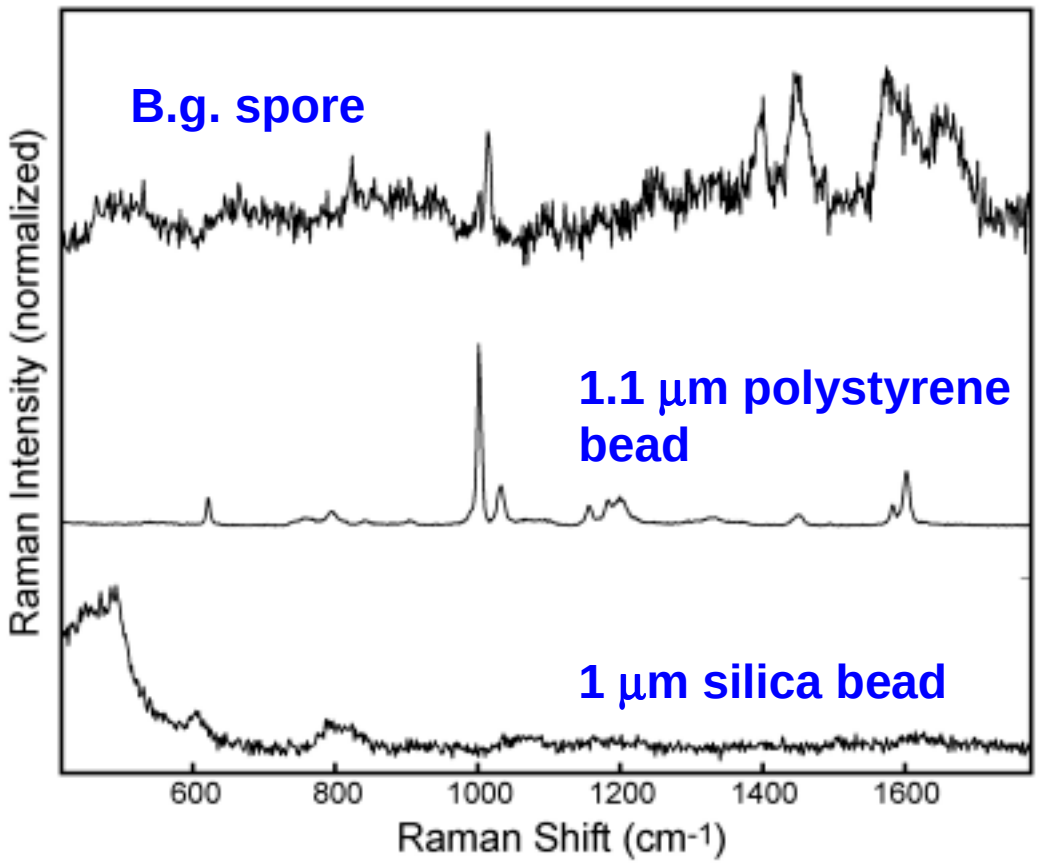


- **A focused laser beam can trap small particles in liquids by radiation pressure**
- **Can study cells in their native environment**
- **Low laser power is sufficient (1 mW)**

Optical Trapping of Cells is Straightforward, Allows for Investigation of Samples in Solution

- Fast, easier sample handling
- No surface—lower background signal
- Can alter solution conditions (pH, media) while holding cell in place

Optical Trapping is Easily Combined with Raman Spectroscopy



- We can trap individual cells and particles and obtain Raman spectra in as little as 5 sec.
- Can use Raman signatures to screen for spores

Summary and Future Directions

Sperm Cells

- **Obtained good localized Raman signal without enhancement**
- **Can quantify relative amounts of protein and DNA in cell heads (potential measure of DNA packaging)**

Bacterial Spores

- **Obtained Raman spectra of individual spores without enhancement**
- **Observed spore-to-spore variability within populations**

Simultaneous Optical Trapping and Raman Spectroscopy

- **Trapped and analyzed spores and other particles in solution**
- **“Reagentless” biodetection method**
- **Try “real time” studies of structural changes in cells (mitosis, viral infection, cell degradation)**

Acknowledgments

- **Co-investigators:** **Chad Talley (CMS)**
  Thomas Huser (CMS)
  James Chan (PAT)
  Steve Lane (PAT)
  Maurice Pitesky (BBRP)
  Shelley Corzett (BBRP)
  Rod Balhorn (BBRP)

- **Funding:** **LLNL Laboratory Directed Research and Development Program**

This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under contract No. W-7405-ENG-48.
UCRL-PRES-150239